sulphates present; and these indicate the origin of the deposit.

To investigate this matter, however, the committee had the water collected in the 8-in. rain gage on the roof of the Meteorological Office analyzed for two or three months. During the month of November the small 8-in. gage collected 900 c.c. of water, the total deposit was 0.445 gramme, the total soluble 0.34 gramme, while in the standard deposit gage, the water collected was 783 liters, total deposit 1.962 grammes, total soluble 0.53 gramme. There was, therefore, a large excess of soluble matter in the water collected in the rain gage. The same result was found in subsequent months, and it was ascertained that the excess of soluble matter was due to metal dissolved from the rain gage.

It was therefore useless to continue the experiment unless the solution of the metal could be prevented. In order to do this the rain gage was given a coating of Duroprene varnish, in the hope that this would prevent the solution of the metal without any contamination of the water.

The result of the analysis of a month's rainfall showed for the 8-in. rain gage a considerably larger proportion of soluble and insoluble matter per liter of water as compared with the standard deposit gage, owing to the varnish yielding to the action of the rain water. It is therefore clear, if observations are to be taken with small gages these must be constructed of something which will not dissolve in the water, and the use of the ordinary copper rain gages is therefore inadmissible.

Alexander McAdie

SPECIAL ARTICLES

THE MECHANISM OF AN ENZYME REACTION AS EXEMPLIFIED BY PEPSIN DIGESTION[1]

One of the most striking peculiarities of living matter is the fact that nearly all the reactions which take place in the organism are due to enzymes. The mechanism of enzyme reactions is therefore very closely connected with the mechanism of the living cell. Many enzyme reactions, however, may be studied in vitro and are therefore amenable to quantitative study. The present paper is an attempt to show that the peculiarities of a typical enzyme reaction, pepsin digestion, may be explained by the accepted laws of chemical reactions and that the apparent divergencies from these laws are due to the fact that the enzyme as well as the protein with which it reacts exist in solution as equilibrium mixtures, consisting, in the case of the protein of ionized and non-ionized protein, and in the case of the pepsin of free and combined pepsin. The influence of the various factors on the digestion are primarily due to changes in these equilibria.

It is well known that enzyme reactions in general have certain peculiarities which distinguish them from ordinary chemical reactions. These may be briefly stated as follows:

1. The final amount of change caused by the enzyme is independent of the amount of enzyme present.

2. The rate of change may or may not be proportional to the concentration of enzyme present.

3. The rate of change is proportional to the substrate concentration in dilute solution but increases less rapidly than the substrate concentration in solution of higher concentration.

4. The amount of substrate decomposed in the same time interval by varying enzyme concentrations is not always proportional to the concentration of enzyme but is often proportional to the square root of this quantity (Schütz's rule).

5. The reaction proceeds most rapidly at a certain definite hydrogen ion concentration.

It has been found in a study of pepsin digestion that the above peculiarities may be quantitatively accounted for on the basis of the following mechanism.

1. The protein reacts with the acid of the

[1] The experimental data on which this paper is based may be found in *J. Gen. Physiol.*, 1918–19, I., 607; 1919–20, II., 113, 465, 471, 595; 1920–21, III., 211.

solution to form an ionized protein salt. The amount of this salt formed is determined by the hydrogen ion concentration of the solution according to the well-known laws governing the reaction of an acid and a weak base.

2. The pepsin is present in the solution, (*a*) as free, probably negatively charged pepsin, and (*b*) in combination with the products of hydrolysis of the protein. These two forms are in equilibrium with each other and their relative concentration depends on the amount of products of hydrolysis present in the solution as demanded by the law of mass action.

3. The reaction takes place between the ionized protein and the free pepsin.

EXPERIMENTAL EVIDENCE FOR THE ABOVE STATEMENTS

Loeb[2] has shown by direct experiment that the protein exists in solution in an equilibrium condition as stated under (1).

Rekelharing and Ringer[3] have shown that purified pepsin in solution is negatively charged. It may be shown by direct experiment that the addition of products of hydrolysis decrease the activity of the enzyme and that the amount of the decrease in the activity can be predicted by the law of mass action.

The validity of the third assumption may best be tested by applying the proposed mechanism to the explanation of the characteristic peculiarities of the reaction outlined under (1 to 5).

1. *Influence of Quantity of Enzyme on the Final Equilibrium.*—Since the free enzyme and the products of hydrolysis are in equilibrium there will always be some active (free) enzyme present no matter how high the concentration of products becomes. The reaction will therefore proceed to approximately the same point irrespective of the amount of enzyme present at the beginning of the reaction. It will be seen, however, that the final equilibrium will depend to a slight extent on the amount of enzyme present since some of the products of hydrolysis are combined with the enzyme.

2. *Concentration of Enzyme.*—If the enzyme solution is pure, the rate of hydrolysis, other factors being constant, will be directly proportional to the concentration of enzyme taken. If the enzyme solution contains products of hydrolysis or other substances with which the enzyme is combined then the rate of hydrolysis will increase more slowly than the concentration of enzyme solution since the amount of free enzyme present becomes relatively smaller the higher the concentration.

3. *Concentration of Protein.*—If the rate of hydrolysis of the protein is proportional to the concentration of *ionized* protein then the rate must increase more slowly than the total protein concentration since the ionization of the protein salt is less in concentrated than in dilute solution.

4. *Schütz's Rule.*—Arrhenius[4] has pointed out that in an equilibrium system, such as exists between free pepsin and the products of hydrolysis, the concentration of one of the reacting molecules or ions becomes inversely proportional to the concentration of the second as soon as the second is present in large excess. That is, the amount of free pepsin present, after the first few minutes of the reaction, is inversely proportional to the amount of products formed. It follows from this that the amount of hydrolysis at any time is proportional to the square root of the time elapsed, which is one form of Schütz's rule.

5. *The Influence of the Hydrogen Ion Concentration.*—It is clear that the more acid is added to the protein the more protein salt will be formed until all the protein is present in the form of protein-acid salt. This salt is practically completely ionized in dilute solution as may be shown by direct measurement

[2] Loeb, J., *J. Gen. Physiol.*, 1918–19, I.; 1919–20, II.

[3] Peckelharing, C. A., and Ringer, W. E., *Z. physiol. Chem.*, 1911, LXXV., 282.

[4] Arrhenius, S., "Quantitative Laws in Biological Chemistry," London, 1915, pp. 36–48.

of the anion concentration by means of concentration cells. A further increase in the amount of acid will now serve to decrease the concentration of protein ions by increasing the concentration of the common anion. The concentration of ionized protein will therefore pass through a maximum which should coincide with the maximum for the rate of digestion. If the ordinary theory of chemical kinetics, on the basis of the law of mass action, be applied to the above system, it may be predicted that:

I. The optimum hydrogen ion concentration for the digestion of the protein must coincide with the hydrogen ion concentration at which the concentration of protein ions and therefore the conductivity due to the protein is at a maximum.

II. The limiting pH for the activity of pepsin on the alkaline side must depend on the isoelectric point of the protein, since this is the point at which the protein first begins to react with the acid.

III. The addition of a salt with the same anion as the acid to a solution already containing the optimum amount of acid will have the same depressing effect on the digestion as the addition of the same amount of anion in the form of acid.

IV. The pepsin should combine with the protein only when the latter is ionized, *i.e.*, pepsin should behave the same as the inorganic anions studied by Loeb.

These predictions have been tested quantitatively and found to be fulfilled. It has also been found by direct experiment that neither the influence of the acidity on the destruction of the enzyme, nor the viscosity of the protein solution can account for the influence of the hydrogen ion concentration on the rate of digestion.

It will be seen that from this point of view pepsin digestion is a chemical reaction in which the pepsin as well as the protein takes part. It is therefore not a catalytic reaction at all in the classical sense. The specificity of the reaction is therefore probably governed by the same conditions that determine the specificity of any chemical reaction, since from a quantitative standpoint each chemical reaction is specific. It may be added that a very similar mechanism was proposed by Stieglitz and his collaborators for the hydrolysis of the imido esters by acid.

It is, of course, impossible at present to apply these results directly to the activities of the living organism since conditions there are much more complex. It is probable, however, that much of the apparent complexity is due to the fact that several processes, each simple in itself, occur simultaneously and thus lead to a complicated result. Dernby's[5] experiments render it probable that the phenomenon of autolysis may be explained in this way.

JOHN H. NORTHROP
ROCKEFELLER INSTITUTE FOR
MEDICAL RESEARCH

KNIPP'S SINGING TUBE

MY colleague, Dr. C. T. Knipp, when constructing a piece of apparatus, found that one of the parts—a glass tube intended for a mercury trap—gave forth a musical sound under the heating action of a gas flame. Following this clue he constructed various modifications of the tube and described them with the interesting results obtained.[1] Inquiry has been expressed concerning the explanation of its action. It occurred to the writer that this explanation might be found in the theory advanced for similar cases where sounds are maintained by heat.[2]

Fig. 1 pictures one type of the tubes tested. It is a resonator with a loop at A and a node at N, so that the distance $ABCN$ constitutes approximately one fourth of the wave-length of the sound given out by the tube when operating.[3] The air surges back and forth at A with the greatest velocity and displacement. From this point the to and fro motion of the

[5] Dernby, K. G., *Biochem. Z.*, 1917, LXXXI., 198.

[1] *Phys. Rev.*, Vol. 15, p. 155, 1920; and other publications.

[2] Rayleigh, "Theory of Sound," Sec. 322. Barton, "Text-Book of Sound," Sec. 265–277.

[3] *Phys. Rev.*, Vol. 15, p. 336, 1920.